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Relationship between circulating anti-Müllerian hormone (AMH) and superovulatory response of high-producing dairy cows

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ABSTRACT

The main objective of this study was to evaluate the relationship between circulating anti-Müllerian hormone (AMH) and superovulatory response of dairy cows. Holstein cows (n = 72) were milked twice daily and housed and fed individually in tiestalls. All animals were synchronized and flushed at 70 ± 3 d in milk (DIM), near peak production (39.6 kg/d). Blood samples for AMH analysis were collected at 3 different stages of a synchronized estrous cycle [at a random stage (40 ± 3 DIM), proestrus (50 ± 3 DIM), and diestrus (57 ± 3 DIM)]. Body weights were measured weekly from calving until embryo collection. Statistical analyses were performed with Proc CORR and Proc GLIMMIX of SAS (SAS Institute Inc., Cary, NC). The 3 AMH samples from individual cows were correlated and not influenced by day of cycle. Surprisingly, AMH tended to be negatively correlated with body weight loss from calving to embryo collection (r = −0.22). More importantly, average AMH was highly associated (r = 0.65) with superovulation response (number of corpora lutea on the day of the flush, CLN), total structures collected (r = 0.48), and total transferable embryos (r = 0.37), but not percentage of fertilized embryos (r = −0.20) or degenerate embryos (r = 0.02). When cows were classified into quartiles (Q) of circulating AMH (Q1 = 0.01 to 82.6 pg/mL; Q2 = 91.1 to 132.5 pg/mL; Q3 = 135.3 to 183.8 pg/mL; Q4 = 184.4 to 374.3 pg/mL), we observed a >2-fold difference between first and fourth AMH quartiles in superovulation response (CLN: Q1 = 12.0 ± 1.5; Q2 = 14.7 ± 2.0; Q3 = 17.2 ± 1.2; Q4 = 25.6 ± 1.5) and embryo production. In conclusion, circulating AMH concentration was strongly associated with superovulation response, and evaluation of AMH could be used to identify cows with greater responses to superstimulation and thus improve efficiency of superovulation programs in dairy cows.

Key words: superovulation, embryo transfer, dairy cow, anti-Müllerian hormone

INTRODUCTION

Recent advances in bovine biotechnology, such as commercially available genomics testing, have allowed for the identification of animals with superior genetics. However, cost-efficient propagation of these superior genetics has been hampered by high variability between animals in response to embryo production techniques, such as superovulation. For example, 30% of the cows are responsible for 70% of the embryos produced during superovulation programs (Bó and Mapletoft, 2014; Hasler, 2014). More-accurate identification of cows with greater embryo production potential could allow for more efficient production of in vitro and in vivo bovine embryos from cows with superior genetics.

Ultrasound determination of antral follicle count has been shown to be correlated with subsequent response to superstimulation in cattle (Singh et al., 2004; Ireland et al., 2007). However, under practical conditions, substantial variation can exist in experience of ultrasound operators, characteristics of ultrasound machines, operator-defined criteria for counting antral follicles, and stage of the follicular wave at time of evaluation (Singh et al., 2004; Burns et al., 2005; Monniaux et al., 2010). A combination of these variables may make it more difficult to reliably select the cows with the greatest capacity for production of embryos under field conditions. Laboratory methods that reliably predict antral follicle numbers and response to superovulation could have substantial value for selection of cows for use in biotechnology protocols or for genomic selection of cows with greater reproductive capacity. In humans, circulating anti-Müllerian hormone (**AMH**) concentrations have been found to be the most informative serum marker for ovarian follicle reserve, largely replac-

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ing other serum markers such as basal FSH testing (Fanchin et al., 2003; Broekmans et al., 2006; Toner and Seifer, 2013). Information is also accumulating in cattle that measurement of circulating AMH concentrations may be the most reliable method for predicting antral follicle numbers (Ireland et al., 2011; Monniaux et al., 2013; Batista et al., 2014).

Anti-Müllerian hormone is a 140-kDa glycoprotein that is a member of the transforming growth factor β (TGFβ) gene/protein family (Knight and Glister, 2006). Anti-Müllerian hormone is exclusively expressed in the gonads, within Sertoli cells of the male and granulosa cells of the female (Monniaux et al., 2013). The physiological function of AMH in adult females is still an area of active research; however, AMH has been shown to modulate early follicular growth and may thereby inhibit excessive number of follicles from entering the growing follicle pool, preventing premature depletion of the ovarian follicle reserve (Durlinger et al., 1999, 2002; Monniaux et al., 2012).

Studies in cattle have confirmed the localization of AMH expression to granulosa cells of small antral growing follicles (Rico et al., 2011) and have defined important aspects of the hormonal regulation of AMH expression and secretion from granulosa cells (Rico et al., 2011; Scheetz et al., 2012; Monniaux et al., 2013). It has been demonstrated in cattle, similar to humans (Nelson, 2013), that circulating AMH concentration is a reliable endocrine marker for the size of the antral follicle population (Ireland et al., 2011; Rico et al., 2011; Monniaux et al., 2012). Studies in the bovine have also evaluated AMH concentrations in peripubertal calves, during the periparturient period, and during the normal bovine estrous cycle (Monniaux et al., 2013). In some studies (Ireland et al., 2011; Rico et al., 2011; Monniaux et al., 2013), small but significant variations in AMH concentrations have been detected under different physiological conditions; however, major variations in AMH concentrations are observed between individuals, demonstrating that cows with a greater antral follicle population have much higher AMH concentrations than cows with a smaller antral follicle population. Thus, the basic studies provide a strong foundation for studies evaluating the practical use of AMH measurements for reproductive biotechnologies in cattle.

Results from the limited number of studies investigating the relationship between circulating AMH and in vivo embryo production following superovulation of dairy cattle have been encouraging. An early experiment (Rico et al., 2009) evaluated 18 Holstein cattle and reported a strong correlation between plasma AMH concentrations and numbers of large follicles after superstimulation ($r = 0.83$) and number of CL after superovulation ($r = 0.64$); however, relationships to embryo numbers were not reported. The largest study (Monniaux et al., 2010) used 45 crossbred Holstein × Normande dairy cows and compared plasma AMH (60–90 DIM) with embryo production during a total of 240 superovulation protocols over a 4-yr time period. In that study, no information was provided on superovulatory response but plasma AMH concentrations were correlated with average numbers of embryos collected per cow ($r = 0.49$) and average number of transferable embryos per donor cow ($r = 0.32$). Two subsequent studies by this same research group (Rico et al., 2012), in 64 superstimulated, nonlactating Holstein cows, found a high correlation of circulating AMH concentrations to number of large follicles following superstimulation ($r = 0.56$) and to corpus luteum (**CL**) numbers after superovulation ($r = 0.43$), although embryo production was not reported. Finally, a study in 34 Japanese Black cows (Hirayama et al., 2012) reported a correlation between AMH concentrations and numbers of transferable embryos ($r = 0.39$). Thus, previous studies are consistent in showing that circulating AMH is a good predictor of superstimulation, superovulation, and embryo production, although only one of the studies combined all 3 responses. In addition, the largest studies were done in nonlactating (Rico et al., 2012), crossbred (Monniaux et al., 2010), or beef (Hirayama et al., 2012) cattle. Given the potential importance of AMH as a predictor of reproductive capacity, further studies are needed on the repeatability of this assay during the estrous cycle and predictability of this assay for superovulatory and embryo production responses in lactating Holstein cattle.

Thus, the objective of this study was to investigate whether circulating AMH could be used as a predictor for superovulation response and embryo production in synchronized, high-producing, lactating Holstein dairy cattle. Concentrations of AMH were evaluated at 3 different times in all cows to determine within-cow repeatability of AMH measurements, as well as any effects of estrous cycle stage on AMH concentrations. Our main hypothesis was that cows with greater circulating AMH would have increased numbers of ovulations after superstimulation with FSH and increased yield of transferable embryos in response to the superovulation protocol.

MATERIALS AND METHODS

Animal Housing and Diets

All procedures were approved by the Animal Care Committee of the College of Agriculture and Life Sciences, University of Wisconsin-Madison. Seventy-two lactating Holstein cows (primiparous = 28, multiparous